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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Inventors:	Eric F. Bernstein
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Examiner:	George, Konata M.
Group Art Unit:	1616
Title:	Compositions and Methods for Prevention of Photoaging

DECLARATION UNDER RULE § 1.131

I, Eric F. Bernstein, hereby declare that:

1. I am a co-inventor of the above-identified application and am most familiar with the subject matter of this application and the research effort occurring in my laboratory located in Philadelphia, Pennsylvania that provided supporting research for this invention.

2. As evidenced by laboratory notebook page 68, attached as Exhibit A hereto, Dr. Echelard, Dr. Forbes and I recorded in writing our idea of using the anti-elastase serine protease inhibitor alpha-1-antitrypsin (or AAT, or API) to prevent skin photoaging. As shown by laboratory notebook page 68, we believed at this time of conception that cream or skin lotions incorporating AAT could be applied topically to prevent photoaging. As also shown by laboratory notebook page 68, we believed that milk from transgenic goats producing AAT would provide a good starting material for this type of cream. We also recorded at laboratory notebook page 68 our intention to demonstrate the viability of this concept *in vitro* in a model of skin photoaging using mouse fibroblasts expressing the human elastin promoter-chloramphenicol acetyl transferase (CAT) construct as well as human subjects.

3. Conception and recordation of this invention at laboratory notebook page 68 occurred prior to November 5, 1998.

4. Further, as evidenced by laboratory notebook pages 1-3 attached hereto as Exhibit B, *in vivo* experiments assessing the ability of the serine proteinase inhibitor alpha-1-antitrypsin to protect against cutaneous photodamage in a transgenic mouse model containing the human elastin promoter linked to a CAT reporter gene were performed. This model is well accepted for its utility in testing compounds that may inhibit cutaneous photodamage in humans and is well established as a model of skin photoaging.

The basis for the acceptance of this animal model for photoaging in humans is as follows. Briefly, humans develop changes in the skin from repeated sun exposure over many years, termed photoaging. These changes consist of fine lines and wrinkles. Underlying these fine lines and wrinkles are changes to the collagen and elastin in skin. The most dramatic change in sun-damaged, or photodamaged, skin is the deposition of massive amounts of abnormal elastic material called 'solar elastosis'. These large clumps of elastic material replace the normally collagen-rich dermis of the skin. Our group and others have shown in the peer-reviewed literature that this elastin results from increased elastin promoter activity, which in turn causes an increase in elastin mRNA, which then in turn leads to elastin deposition in photodamaged skin. Thus, elastin promoter activation is one of the first events leading to photoaged skin.

Elastin promoter activation in our model has also been shown in the peer-reviewed literature to correlate with DNA damage. Skin cancer and sunburn result from DNA damage as well, and these events are believed to correlate with the results shown by our model as well.

Thus, using our patented model that utilizes transgenic mice containing the human elastin promoter-CAT construct, we can model photodamage and test compounds thought to prevent photoaging, sunburn and skin cancer.

In the experiments depicted in laboratory notebook pages 1-3, milk samples from two transgenic goats, D-161 and D-174 each producing alpha-1-antitrypsin in different concentrations, were formulated with various carriers Lubriderm, Aquaphor and propylene glycol at ratios of 1:1, 1:2, 1:3, 1:4 and 1:5. A 1:6 ratio of propylene glycol to milk was also prepared. Formulations of Aquaphor and milk solidified. Formulations of propylene glycol and milk did not get into the skin very easily until ratios of 1:5 and 1:6 were applied. Formulations of Lubriderm and milk had no problems. Accordingly, 2 mg/cm^2 of a 1:1 Lubriderm:milk formulation from goat D-161 or goat D-174 was applied to the backs of to the above-described transgenic mice. Controls for this experiment included untreated mice and untreated mice exposed to solar-simulating light. In this experiment, both 10 and 20 minimal erythema doses (MEDs) of solar-

simulating light were administered and samples tested. Ten MEDs of light was not enough to increase promoter induction beyond a 3.1-fold increase, and thus compounds could not be adequately tested with this light dose. Using 20 MEDs of light resulted in a 21.5-fold increase in elastin promoter activity. This light dose was optimal for measuring a possible protective effect of the test compounds. Promoter induction was decreased from a 21.5-fold increase in the controls treated with solar-simulating light to a 11.3-fold and a 16.4-fold increase in the D-161 and D-174 treated groups, respectively. Thus, milk containing alpha-1-antitrypsin was capable of protecting against elastin promoter damage. Thus, this experiment was indicative of the ability of serine protease inhibitors to protect the skin against photoaging, sunburn and skin cancer.

5. The experiment described in the attached laboratory notebook pages 1-3 were performed and the results obtained under my direct supervision prior to November 5, 1998.

6. Additional experiments, the results of which are attached hereto in Exhibit C were also performed using milk from transgenic goats containing alpha-1-antitrypsin and control goats' milk. The milk samples were mixed into a cream and rubbed onto the back of mice containing the human elastin promoter-CAT construct. Mice were treated three times daily with these creams and then subjected to solar-simulating light. Mice were then euthanized, the skin harvested, and CAT protein concentrations indicative of elastin promoter activation were determined. The milk containing alpha-1-antitrypsin demonstrated the most protection against photoaging by blocking the light-induced promoter elevation. Milk alone blocked some of the promoter activation, as well. Thus, this experiment was indicative of the ability of serine protease inhibitors and milk to protect the skin against photoaging, sunburn and skin cancer.

The results from these experiments are depicted in Figure 1, attached hereto as Exhibit C.

7. The experiments set forth in the Figure 1 of Exhibit C were performed under my direct supervision prior to November 5, 1998.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of

Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Eric F. Bernstein, M.D.

3/12/03
Date

Project No.______ CONFIDENTIAL PROPERTY OF GENZYME CORPORATION

68 Book No.______ TITLE ______

From Page No.__ Thursday [redacted]

EXHIBIT A

Patent disclosure

This is to keep a record of the following idea: Derived from a conversation in Philadelphia, after Nov, [redacted]

The Anti Elastase activity of α1-Antitrypsin (or AAT, or API) could be useful as a mechanism of preventing skin photoaging. Hence, Cream or Skin lotions incorporating AAT could be applied topically to prevent photoaging. Milk from the transgenic goats producing ~~AAT~~ AAT would be a perfect raw material for this type of cream. The viability of this idea could be tested "in vitro" on mouse fibroblasts expressing an elastin-CAT construct in the advent of a positive result, a cream containing AAT could be tested with human subjects.

Witnessed & Understood by me, [signature]

Date [redacted]

Invented by Eric Bernstein, Yann Echelard, Paul-Don Forbes

Date [redacted]

Recorded by [signature]

EXHIBIT B

Received sample from [illegible] Eckhard. Store in 4°C (Lower Bin)
2 × 50ml conical tubes - T-440 D-161 (10-31-97)
2 × 50ml conical tubes - 472-95 D-174 (10-31-97)
1 × 15ml conical tube - Alpha-1-Proteinase Inhibitor 6.8 mg/ml 472-78-8082

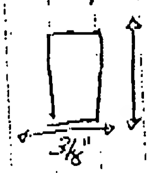
[redacted]

Mix Vitamin D milk with Aquaphor - (from clinic)
Lubriderm - Fragrance Free
Propylene Glycol - SIGMA P-1009 (500ml)

Lubriderm : Milk	Aquaphor : Milk	Propylene Glycol : Milk
1:1 ✓	1:1 x	1:1 x
	1:2 x	1:2 x
	1:3 x	1:3 x
	1:4 x	1:4 ?
	1:5 x	1:5 ?
		1:6 ✓

Heating in 65°C bath for 10-15 min helps to dissolve, but Aquaphor tends to solidify. Propylene Glycol did not get into skin very easy until 1:5, 1:6. Lubriderm @ 1:1 with milk = No problem

2 litter of pups born [redacted]. Use 2mg/cm² of 1:1 mixture of Lubriderm + D-161 and Lubriderm + D-174



$= 0.2813\ in^2 = 0.7144\ cm^2$

$2\ mg/cm^2 \Rightarrow \frac{2mg}{cm^2} \cdot 0.7144\ cm^2 = 1.4288\ mg = 0.0014\ g$

10 MED UVB = 35m56s

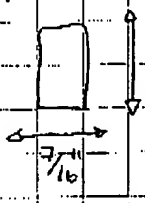
1, 2	untreated controls	(cut tail)
3, 4	untreated UVB only	(nothing)
5 →	UVB + D-161	(F.R)
6 →	UVB + D-174	(B.L)

20 MED UVB = 1h11m51s

1, 2	untreated controls	(cut tail)
3, 4	untreated UVB only	(nothing)
5 →	UVB + Lubriderm	(F.L)
6 →	UVB + D-161	(F.R)
7 →	UVB + D-174	(B.L)

2

[redacted] Day 2 of 3 - 2mg/cm² of 1:1 Lub + D-161 + D-174 [redacted]

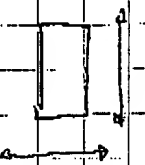


= 0.3828 in²
= 0.9723 cm²

2 mg/cm² · 0.9723 cm² = 1.944 mg
= 0.002 g

Heat solution (cream) @ 42° for 12-15 minutes

[redacted]



(1.0625)(0.5) = 0.5313 in²
= 1.3494 cm²
(2 mg/cm²)(1.3494 cm²) = 2.6988 mg
= 0.003 g

Day 3 of 3 2 mg/cm² 1:1 Lub + D-161 + D-174 [redacted]

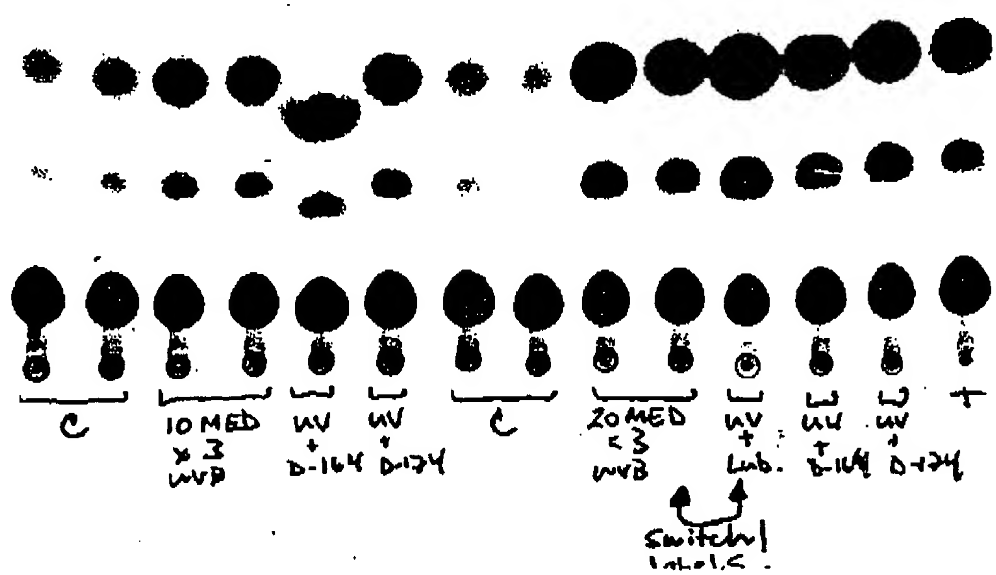
[redacted] Sacrifice Mice
Homogenize Tissue
Extract Supernatant (Protein containing)
Run Spect @ 595nm B.C. Assay

tube	description	absorbance (2µl)	prot. extract. (µl)	Lysis Buffer deH2O (µl)
1	10-cont	0.455	73.4	26.6
2	10-cont	0.398	83.9	16.1
3	10 MED	0.464	72.0	28.0
4	10 MED	0.408	81.9	18.1
5	10 MED + D-161	0.396	84.3	15.7
6	10 MED + D-174	0.476	70.2	29.8
7	20-cont	0.509	65.6	34.4
8	20-cont?	0.539	62.0	38.0
9	20 MED?	0.461	72.5	27.5
10	20 MED	0.416	80.3	19.7
11	20 MED + Lub	0.334	100.0	0.0
12	20 MED + D-161	0.368	90.8	9.2
13	20 MED + D-174	0.349	95.7	4.3

Run until [illegible] w/ a addition [illegible] [redacted]

Load Plate & Run 95 Chloroform : 5 MeOH for 50 minutes

Develop film



		2X 10 MED	3X 20 MED	
2	C	1.0	1.0	
2	UV	3.1 3.9%	21.5	15.6 / 27.8
2	UV + D-164	4.2	11.3	47.4%
2	UV + D-174	[illegible]	16.4	[illegible]%
2	UV + Lub	-	9.3	R [illegible] - 56.7%

10

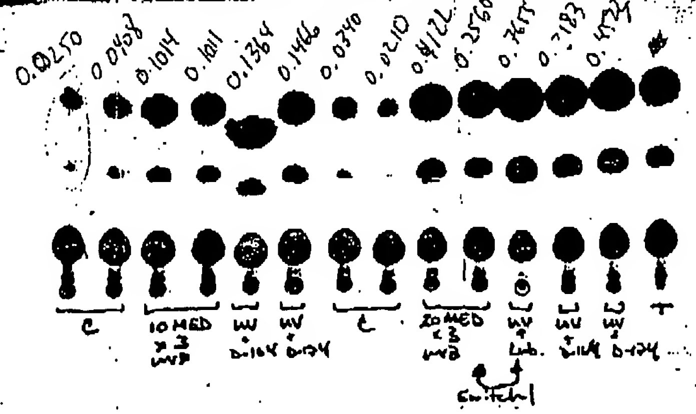


EXHIBIT C

Serine Protease Inhibitors

Effect of alpha-1-antitrypsin on CAT activity

SSR=solar simulating radiation
α1=alpha-1-antitrypsin